Phenotypic and genotypic alterations characterize patients bearing plasma cell dyscrasias with a high M-component

C. Greco*, F. Ameglio†, F. Alvino*, A. Mosiello*, A. M. Cianciulli*,
I. Venturo†, G. Del Monte‡, M. Giampaolo¶, A. W. Tong§ and
G. M. Gandolfo*

**Clinical Pathology Laboratory, Regina Elena Cancer Institute of Rome, †Clinical Pathology Laboratory, S. Galliciano Hospital, ‡2nd Medical Oncology Dept, Regina Elena Cancer Institute, Rome, Italy; ¶Medical Oncology Dept. of the Anagni Hospital, Anagni, Italy; and §The Baylor University, A. Sammons Cancer Center, Dallas, Texas, USA*

(Received 28 October 1998; revision accepted 30 March 1999)

Abstract. As at present only a long-term follow-up can fully determine whether monoclonal gammopathies of undetermined significance (MGUS) will evolve into multiple myeloma (MM), this study attempted to identify other variables connected with the amount of monoclonal component (MC), generally considered as the most reliable marker of malignant evolution. Thirty-four MGUS subjects showing a high MC (≥ 15.0 g/l) but without clinical evidence of MM (MGUS group b), were characterized for their phenotypic and genotypic profile by comparing them either with 40 MM patients or with 24 subjects affected by a benign form of monoclonal gammopathy (MGUS group a) according to the standard criteria. In addition to the usual laboratory markers, the levels of expression of a panel of CD membrane subsets were measured on B and T lymphocytes. Also, the serum level of the p53 mutant protein and the structural alterations of the *c-myc* oncogene were evaluated. The results show that for MGUS group b patients, an increased M-protein was accompanied by significantly increased levels of peripheral blood CD3⁺ T cells and oncogenetic aberrations in *c-myc*. Since a high serum MC level seems to indicate a greater likelihood of malignant transformation for MGUS patients, these findings suggest that this relationship may be a result of the concomitant alterations observed at a phenotypic and genotypic level. Such alterations may be potentially useful as surrogate markers for the transition of benign to malignant (MM) plasma cell dyscrasia.

INTRODUCTION

Plasma cell dyscrasias (PCD), which include monoclonal gammopathies of undetermined significance (MGUS) and multiple myeloma (MM), constitute the most common immunoglobulin disorders. They generate clinical interest primarily owing to their poor responsiveness to therapy and increasing incidence (Ganjoo & Malpas 1995). The stable

Correspondence: Dr Claudia Greco, Clinical Pathology Department, Regina Elena Cancer Institute, 291, v.le Regina Elena, 00161 Roma, Italy.

condition of MGUS patients associated with a low level of the M-component was generally considered to be benign. However, it has now been established that a portion of MGUS cases (up to 25%) evolve into a malignant monoclonal gammopathy (MM) (Kyle 1992).

Since a long-term follow-up alone can verify the malignant transformation of MGUS, up until now only retrospective studies have been carried out to try to understand this transformation. Although results have been conflicting, perhaps as a result of the clinico-biological heterogeneity of PCD, M-component (MC) has been identified as the most reliable marker in signalling the transition from MGUS to MM (Aguzzi *et al.* 1992; Baldini *et al.* 1996). Indeed, the MC level has allowed the stratification of MGUS patients according to different risk groups (Møller-Petersen & Schmidt 1986).

The purpose of the present study was to elaborate a 'biological identikit' of nonmyelomatous MGUS patients showing a high MC (≥ 15.0 g/l). Specifically, the study attempted to identify eventual alterations involving parameters other than the serum paraprotein level in these patients.

To this aim, the phenotypic and genomic profile of high MC-MGUS subjects was characterized by comparing them with either benign MGUS (Kyle 1984) or MM patients. A panel of conventional phenotypic markers was investigated together with immunophenotypic parameters (CDs). In addition, the serum level of the p53 mutation form (p53 M) was evaluated, since a potential involvement of this anti-oncogene product in haematological disorders has already been postulated (Imamura, Miyoshi & Koeffler 1994).

Finally, MGUS and MM patients were examined for *c-myc* structural alterations occurring at the level of peripheral blood cell lymphocytes (PBCL). *c-myc* is a member of cellular proto-oncogenes implicated in the control of normal cell growth by mediating the transition from quiescence to proliferation. Constitutional activation of this gene has mainly been demonstrated in haematological diseases with high mitotic indices (Croce 1987); however, more recently evidence has accumulated to show that *c-myc* also plays a role in the malignant transformation of PCD (Greil *et al.* 1991).

MATERIALS AND METHODS

Patients

Ninety-eight consecutive patients with monoclonal gammopathy entered this study and were subdivided into the following groups: MGUS group a, 24 subjects affected by a benign form of MGUS according to standard criteria (Kyle 1984); MGUS group b, 34 MGUS subjects showing a MC ≥ 15.0 g/l in the absence of clinical signs of malignant disease (i.e. plasma cell percentage $\leq 10\%$ and RX negative for osteolytic lesions); and MM, 40 patients diagnosed according to Durie & Salmon's (1975) criteria and classified as stage I or II when analysed at the onset of the disease before receiving therapy (see Table 1 for the main clinical features). The normal controls were 20 healthy subjects (10 females and 10 males comparable for median age with the patient groups) whose blood samples were processed in the same way as the pathological samples and served as reference for the phenotypic and genotypic profile of the pathological subjects. The MC cut-off level for the MGUS group b was chosen as ≥ 15.0 g/l since this level has frequently been associated with a significant propensity towards progression to MM (Blade *et al.* 1992; Baldini *et al.* 1996). Furthermore it represented the highest cut-off value of M-paraprotein found in nonmyelomatous MGUS patients admitted to the authors' hospital.

No significant differences were observed for sex distributions or age median values within the three groups of patients.

Table 1. Main features of MGUS and MM patients

	MGUS a	MGUS b	MM
Patients (n)	24	34	40
M/F	13/11	20/14	25/15
Age (years)			
Median	56	65	66
(range)	(50–77)	(45–84)	(37–86)
Isotype (H chain)			
IgG	24	23	29
IgA	–	10	11
IgM	–	1	–
Isotype (L chain)			
K	16	19	28
λ	8	15	12
Bence Jones			
Yes	–	2	29
No	24	32	11

Serum parameters

In addition to standard laboratory tests, the following parameters were evaluated: M-protein (typing by immunofixation and quantification by densitometry (Beckman; Brea, CA, USA); β_2 -microglobulin (β_2 M; MEIA, Abbott, Chicago, IL, USA) (normal range 0.7–2.4 mg/l); and interleukin (IL)-6 (enzyme-linked immunosorbent assay (ELISA), Medgenix (Brussels, Belgium); (normal range 0–6 pg/ml)). The serum level of p53 mutation form (p53 M) was determined according to an ELISA assay (Oncogene Science, Mondale, NY, USA) which uses two monoclonal antibodies: pAb 1801 and pAb 421 specific for human and mouse p53 protein epitopes. As previously reported, these antibodies detect either mutated or wild type p53: however, owing to the markedly longer half life of mutated p53 compared to wild form, only mutated proteins accumulate to detectable levels in biological specimens associated with an activated *p53* oncogene (Greco *et al.* 1994).

Immunophenotyping

Quantitative and phenotypic evaluation of PBCL was performed by flow cytometric analysis (Cytoron Absolute Flow Cytometer, Ortho Diagnostic Systems, Rantan, New Jersey, USA) by standard procedure (Knowles *et al.* 1992). The panel of monoclonal antibodies included: CD19, CD3, CD4, CD8, CD56 and CD3HLA-DR purchased from Ortho Diagnostic Systems.

Southern blot analysis

DNA was prepared from isolated lymphocytes following standard procedure (Miller & Phillips 1969). Ten milligrams of genomic DNA were subjected to electrophoresis in 0.8% agarose gel and blotted on Hybond N+ filter (Amersham International, Amersham, UK). Southern blots were hybridized with a *c-myc* probe (3rd exon, 1.4 kb fragment; Oncor, MD, USA), labelled with digoxigenin–dUTP according to the manufacturer's protocol (random priming system, Boehringer Mannheim, Mannheim, Germany). The levels of amplification in individual cases were determined by densitometric comparison (LKB 2202 Ultrascan Laser Densitometer, Bromma, Sweden) of the intensity signals of *c-myc* from normal and pathologic PBCL. Filters hybridized with the *c-myc* probe were then boiled and reprobed

with a β -actin probe as an internal control. The criteria followed to assess gene amplification was according to Peltomäki, Alftan & De La Chapelle 1991.

Statistical analyses

The Kruskal–Wallis test was used to simultaneously compare the values of serum parameters or the expression of each lymphocyte antigen analysed in the four groups: controls, MGUS group a, MGUS group b and MM. The Student's *t*-test or the Mann–Whitney *U*-test were used for comparative analysis between pairs of groups of interest (MGUS group a vs. MGUS group b) and (MGUS group b vs. MM), as specified. The chi-square test was employed to compare the percentages of *c-myc* alterations in the three groups of patients.

Correlation between CD3-HLADR⁺ cells and MC amounts was assessed using Spearman's correlation coefficient.

RESULTS

Serum markers

The serum levels of different biochemical markers, determined either on MGUS (group a and b) or on MM patients are reported in Table 2. As expected, the mass-related parameter β_2 M significantly differs in the three groups of patients ($P < 0.01$) according to the different MC levels. The serum IL-6 level too elicited a statistically significant difference between MGUS (low and high MC) and MM groups ($P < 0.0001$), as a sign of increased cell proliferative capacity. Conversely, the concentration of the p53 protein in the serum of MGUS (groups a and b) and MM patients was found to be similar.

Immunophenotyping

To investigate the behaviour of the main subsets of PBL from both MGUS and MM patients, a FCM analysis was performed and the immunophenotypic features were compared with those of healthy controls.

As reported in Table 3, a statistically significant difference between the various groups was shown for both B and T lymphocytes. In the latter case, a marked increase in the percentage of the cells positive for CDs is observed when comparing low with high MC-MGUS groups (a and b, respectively). This is particularly evident for the CD8 subset.

A significantly different behaviour was shown by lymphocytes positive for CD19 antigen, whose absolute number markedly decreased when passing from MGUS group a to MGUS group b. No difference was found between MGUS group b and MM for the same antigen. As far as lymphocytes positive for CD16/CD56 are concerned, a similar behaviour to that of CD19⁺ cells was shown.

Table 2. Serum markers of MGUS and MM patients (mean $\pm$ sd)

Parameters	Control	MGUS a	MGUS b	MM	* <i>P</i>	† <i>P</i>	‡ <i>P</i>
MC (g/l)	–	10.5 $\pm$ 2.7	19.5 $\pm$ 4.3	28.4 $\pm$ 9.6	< 0.0001	< 0.0001	< 0.0001
β_2 M (μ g/l)	1.8 $\pm$ 0.2	1.9 $\pm$ 0.7	2.4 $\pm$ 1.3	4.2 $\pm$ 2.7	< 0.01	NS	< 0.05
IL-6 (pg/ml)	1.7 $\pm$ 1.2	5.7 $\pm$ 4.7	7.4 $\pm$ 4.7	23.4 $\pm$ 5.2	< 0.0001	< 0.05	< 0.0001
P53 M (ng/ml)	0.07 $\pm$ 0.005	0.08 $\pm$ 0.05	0.1 $\pm$ 0.07	0.12 $\pm$ 0.09	NS	NS	NS

MC, monoclonal component. β_2 M, β_2 -microglobulin. IL-6, interleukin-6. p53 M, p53 mutant.

**P*: KruskalWallis test; †*P*: comparison between MGUS group a and MGUS group b and

‡*P*: comparison between MGUS group b and MM (Student's *t*-test).

Table 3. Peripheral blood lymphocyte distribution in MGUS and MM patients

Lymphocytes	Controls	MGUS a	MGUS b	MM	*P	†P	‡P
CD19	0.21 (0.09–0.45)	0.16 (0.03–0.72)	0.07 (0.01–0.59)	0.08 (0.03–2.02)	< 0.005	< 0.0001	NS
CD3	1.36 (0.60–1.97)	1.45 (0.74–1.99)	1.85 (0.49–3.22)	0.99 (0.33–14.7)	< 0.005	< 0.01	< 0.01
CD4	0.78 (0.39–1.14)	0.85 (0.45–1.22)	0.99 (0.24–1.81)	0.64 (0.02–6.01)	< 0.05	NS	< 0.05
CD8	0.57 (0.22–1.07)	0.40 (0.28–0.81)	0.68 (0.19–1.96)	0.45 (0.08–8.18)	< 0.05	< 0.05	< 0.05
CD3-HLADR	0.09 (0.02–0.27)	0.19 (0.04–0.36)	0.43 (0.09–1.54)	0.24 (0.09–2.16)	< 0.001	< 0.0001	0.05
CD16-CD56	0.17 (0.04–0.60)	0.29 (0.05–1.07)	0.13 (0.03–0.47)	0.20 (0.04–2.15)	< 0.05	< 0.01	NS

Values are expressed as absolute number of positive cells (median and range $\times 10^9/L$). *P (Kruskal–Wallis test) evaluates the differences within the four groups. †P (Mann–Whitney test) indicates the differences between MGUS group a and MGUS group b; ‡P (Mann–Whitney test) indicates the differences between MGUS group b and MM group.

Interestingly enough, in the case of the CD3-HLADR antigen, the increase in the percentage of positive cells observed between low and high MC-MGUS groups (a and b, respectively), was reflected by the positive correlation with the M-component amount (Figure 1).

c-myc alterations

Since the *c-myc* gene has been shown to be altered in malignant PCD (Greil *et al.* 1991, Humphries & Williams 1993), Southern blot analysis was performed on MGUS subjects (group a and b) and MM patients in order to investigate the presence of structural abnormalities of this gene. As reported in Table 4, 10 out of 18 (55.5%) and five out of 16 (31.2%) *c-myc* alterations were found in MM and MGUS group b DNA samples, respectively (Figure 2). In addition, a statistically significant difference was found in MC levels between patients of MGUS group b eliciting or not eliciting *c-myc* gene alterations ($P = 0.01$).

None of the benign MGUS analysed manifested *c-myc* gene alterations.

Figure 1. Correlation between the percentage of CD3-HLADR positive cells and M-component concentration (g/l) in PCD patients ($r = 0.50$; $P = 0.0003$, Spearman's correlation test).

Table 4. *c-myc* alterations related to M-component concentration

Groups	MGUS a	MGUS b	MM	χ^2
Subjects (n)	12	16	18	
M-component (g/l) [§]				
A	–	20.0 $\pm$ 2.8**	27.8 $\pm$ 14.3 [†]	
WA	–	15.8 $\pm$ 1.6	23.6 $\pm$ 3.4	
Total	9.7 $\pm$ 2.2	17.2 $\pm$ 2.3	26.6 $\pm$ 11.1	
<i>c-myc</i> amplifications n (%)	0 (0)	5 (31.2)	10 (55.5)	$P = 0.006\ddagger$

** $P = 0.01$ (Student's *t*-test) refers to the comparison between MC values of MGUS group b with *c-myc* amplifications (A) or without *c-myc* amplifications (WA). The same comparison was applied to MM patients ($\dagger P = 0.5$); $\ddagger P$ (chi-square test) referred to the comparison of frequencies of *c-myc* alterations within the three groups of patients. [§]Mean $\pm$ sd.

DISCUSSION

The aim of the present paper was to characterize MGUS patients showing a high MC, according to their phenotypic and genotypic profile, rather than to predict their potential malignant evolution. The data clearly show that statistically significant variations both at phenotypic and genotypic levels occur in the three groups (MGUS group a, MGUS group b, MM), in concomitance with a MC increase. Owing to the diagnostic and prognostic role of IL-6 for PCD diseases (Bataille *et al.* 1989), the positive gradient observed in the serum cytokine level when proceeding from benign MGUS to MM through high MC-MGUS groups could represent the intrinsic malignant potential of plasma cells, progressively expressed through the various steps of PCD disorders.

p53 protein appears to take only a marginal role, if any, as a discriminating marker between the various groups of MGUS and MM. This can be explained by the fact that the present MM patients, at the time of the study, were at stages I or II of the disease while p53 mutations have

Figure 2. *c-myc* gene amplifications in MGUS group b. DNA (10 μ g) from PBCL was digested with *Eco*RI, fractionated by electrophoresis on a 0.8% agarose gel and processed as described in *Material and methods*. N, normal donors. M1, M2, M3, M4, M5, MGUS patients.

been associated with more advanced and clinically aggressive forms of MM (Neri *et al.* 1993). The phenotypic analysis of differentiation antigens has proven valuable for diagnostic or prognostic purposes in haematological diseases, such as leukaemia or lymphoma (Foon & Todd 1986). However, data have been conflicting on the immunological phenotype of MGUS and MM.

Previous studies have reached contradictory results on the amount of peripheral blood B lymphocytes (CD19⁺) in MM patients. The present data show that the absolute number of CD19⁺ cells is significantly lower in MM patients compared to healthy controls. This finding cannot depend on the age of patients, because there is no significant difference for the age values between the various groups ($P = 0.06$). In addition, the evidence that CD19⁺ cells decrease in MM patients is questionable (Kay *et al.* 1995). However, a marked decrease of this antigen is already evident in peripheral B lymphocytes of MGUS group b. Owing to the complexity and heterogeneity in the number of circulating CD19⁺ B cells detected by FCM in MM patients (Kay *et al.* 1997), further studies are needed to examine the nature of this lymphocyte population in the high MC-MGUS group.

A regulatory role played by T cell populations in PCD pathologies has been postulated by some authors (San Miguel, Caballero & Gonzales 1985). Nevertheless, it is still unclear whether changes in these lymphocytic subsets represent a response to the monoclonal gammopathy or their relation is purely coincidental.

The results of the present paper suggest that T cell alterations may be a response to the MC increase, owing to the particular behaviour of the various CD subsets, when passing from low MC-MGUS to high MC-MGUS group (Table 3). This is suggestive that the M-component and the CD3-HLADR antigen are positively related (Figure 1). Such a specific immune stimulation could be induced by the phenotypic changes of the plasma cell clone which is undergoing transformation (increases in M-paraprotein, β_2 M and IL-6).

The concomitant decrease of the CD16/CD56 antigen observed in MGUS group b, other than in MM, seems to confirm this hypothesis. In fact, the increase of T lymphocyte subsets could represent an attempt to suppress the expansion of the plasma cell clones escaping the control of natural killer cells. Based only on these immunophenotypic data, this hypothesis is merely suggestive. Nevertheless, it is worthwhile to examine thoroughly this point in order to extrapolate these results in terms of disease pathophysiology.

The involvement of apparently normal PBCL in the pathogenesis of MM disease has been suggested by several authors. Indeed these cells could represent the true precursors of malignant plasma cells (Van Riet *et al.* 1989, Pilarski *et al.* 1996).

Given this and the difficulty of obtaining informed consent by MGUS patients for bone marrow specimens, the *c-myc* gene analysis was carried out on DNA from PBCL.

Here, *c-myc* alterations are reported not only in MM patients but also in high MC-MGUS patients. This finding confirms the association of *c-myc* abnormalities with tumour cell mass, as described by other authors (Greil *et al.* 1991). It also shows that a genetic transformation is already apparent in this group of MGUS patients. Fluorescence *in situ* hybridization (FISH) cytogenetic analysis currently being performed in the authors' laboratory has in fact shown that aneusomy of chromosomes 1,8 and 17 occurs in the MGUS group b (Cianciulli *et al.* 1997).

In conclusion, a high proliferative activity (assessed by serum IL-6 levels) together with changes in immunophenotypic and genotypic profile characterize MGUS patients showing a high MC, compared to MGUS subjects with a constantly low MC level.

Given these results, it can be hypothesized that high MC-MGUS patients are more subjected to malignant transformation than low MC-MGUS ones. This hypothesis, however, needs to be verified. It is also necessary to establish whether MC changes are preceded by changes in IL-6,

increased levels of CD3⁺ T cells and *c-myc* alterations. If so, these parameters might be earlier and consequently better risk markers than the singular use of MC for MGUS disease.

REFERENCES

- AGUZZI F, BERGAMI MR, GASPARRO C, BELLOTTI V, MERLINI G. (1992) Occurrence of monoclonal components in general practice: clinical implications. *Eur J. Haematol.* **48**, 192.
- BALDINI L, GUFFANTI A, CESANA BM *et al.* (1996) Role of different hematologic variables in defining the risk of malignant transformation in monoclonal gammopathy. *Blood* **87**, 912.
- BATAILLE R, JOURDAN M, ZHANG XG, KLEIN B. (1989) Serum levels of interleukin-6, a potent myeloma cell growth factor, as a reflection of disease severity in plasma cell dyscrasias. *J. Clin. Invest.* **84**, 2008.
- BLADE J, LOPEZ-GUILLERMO A, ROZMAN C *et al.* (1992) Malignant transformation and life expectancy in monoclonal gammopathy of undetermined significance. *Br. J. Haematol.* **81**, 391.
- CIANCHIULLI AM, COLETTA AM, PESCATORE B *et al.* (1997) Monoclonal gammopathy and multiple myeloma: incidence of chromosomal aneuploidy detected by interphase fluorescence *in situ* hybridization. *Eur J. Histochem.* **14**, 157.
- CROCE C. (1987) Role of chromosome translocations in human neoplasia. *Cell* **49**, 155.
- DURIE BGM, SALMON SE. (1975) A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer* **36**, 842.
- FOON KA, TODD RF. (1986) Immunologic classification of leukemias and lymphomas. *Blood* **68**, 1.
- GANJOO RK, MALPAS JS. (1995) Plasmacytoma. In: MALPAS JS, BERGSAGEL DE, KYLE RA, eds. *Myeloma: Biology and Management*. Oxford: University Press, 463.
- GRECO C, GANDOLFO GM, MATTEI F *et al.* (1994) Detection of *c-myc* genetic alterations and mutant p53 serum protein in patients with benign and malignant colon lesions. *Anticancer Res.* **14**, 1433.
- GREIL R, FASCHING B, LOIDL P, HUBER H. (1991) Expression of the *c-myc* proto-oncogene in multiple myeloma and chronic lymphocytic leukemia: an *in situ* analysis. *Blood* **78**, 180.
- HUMPHRIES JE, WILLIAMS ME. (1993) Detectability of immunoglobulin and *c-myc* gene rearrangements in multiple myeloma. *Br. J. Haematol.* **83**, 173.
- IMAMURA J, MIYOSHI I, KOEFFLER HP. (1994) P53 in hematologic malignancies. *Blood* **84**, 2412.
- KAY NE, LEONG T, KYLE RA *et al.* (1997) Circulating blood B cells in multiple myeloma: analysis and relationship to circulating clonal cells and clinical parameters in a cohort of patients entered on the Eastern Cooperative Oncology Group phase III E9486 Clinical Trial. *Blood* **90**, 340.
- KAY NE, OKEN MM, BONE N *et al.* (1995) Circulating CD19+ blood cell levels in meloma. *Blood* **86**, 4000.
- KNOWLES DM, CHADBURN A, INGHIRAMI G. (1992) Immunophenotypic markers useful in the diagnosis and classification of hematopoietic neoplasms. In: KNOWLES DM, ed. *Neoplastic Hematopathology*. Baltimore: Williams & Wilkins, 73.
- KYLE RA. (1984) "Benign" monoclonal gammopathy. *JAMA* **251**, 1849.
- KYLE RA. (1992) Diagnostic criteria of multiple myeloma. *Hematol. Oncol. Clin. North America* **6**, 347.
- MILLER R, PHILLIPS R. (1969) Separation of cells by velocity sedimentation. *J. Cell Physiol.* **73**, 191.
- MØLLER-PETERSEN J, SCHMIDT EB. (1986) Diagnostic value of the concentration of M-component in initial classification of monoclonal gammopathy. *Scand. J. Haematol.* **36**, 295.
- NERI A, BALDINI L, TRECCA D, CRO L, POLLI E, MAIOLO AT. (1993) P53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. *Blood* **81**, 128.
- PELTOMÄKI P, ALFTHAN O, DE LA CHAPPELLE A. (1991) Oncogenes in human testicular cancer. DNA and RNA Studies. *Br. J. Cancer* **63**, 851.
- PILARSKI LM, MASELLIS-SMITH A, SZCZEPEK A, MANT MJ, BELCH AR. (1996) Circulating clonotypic B cells in the biology of multiple myeloma: speculations on the origin of myeloma. *Leuk. Lymphoma* **22**, 375.
- SAN MIGUEL JF, CABALLERO MD, GONZALES M. (1985) T-cell subpopulations in patients with monoclonal gammopathies. *Am. J. Hematol.* **20**, 267.
- VAN RIET I, HEIRMAN C, LACOR P, DE WAELE M, THIELEMANS K, VAN CAMP B. (1989) Detection of monoclonal B lymphocytes in bone marrow and peripheral blood of multiple myeloma patients by immunoglobulin gene rearrangement studies. *Br. J. Haematol.* **73**, 289.